

AUTORADIOGRAPHIC STUDIES OF SYNTHESIS AND INTRACELLULAR MIGRATION OF GLYCOPROTEINS IN THE RAT ANTERIOR PITUITARY GLAND

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ABSTRACT

The incorporation of [^3H]fucose in the somatotrophic and gonadotrophic cells of the rat adenohypophysis has been studied by electron microscope autoradiography to determine the site of synthesis of glycoproteins and to follow the migration of newly synthesized glycoproteins. The pituitaries were fixed 5 min, 20 min, 1 h, and 4 h after the *in vivo* injection of [^3H]fucose and autoradiographs analyzed quantitatively. At 5 min after [^3H]fucose administration, 80–90% of the silver grains were localized over the Golgi apparatus in both somatotrophs and gonadotrophs. By 20 min, the Golgi apparatus was still labeled and some radioactivity appeared over granules. At 1 h and 4 h, silver grains were found predominantly over secretory granules. The kinetic analysis showed that in both protein-secreting cells (somatotrophs) and glycoprotein-secreting cells (gonadotrophs), the glycoproteins have their synthesis completed in the Golgi apparatus and migrate subsequently to the secretory granules. It is concluded from these *in vivo* studies that glycoproteins which are not hormones are utilized for the formation of the matrix and/or of the membrane of the secretory granules. The incorporation of [^3H]fucose in gonadectomy cells (hyperstimulated gonadotrophs) was also studied *in vitro* after pulse labeling of pituitary fragments in medium containing [^3H]fucose. The incorporation of [^3H]fucose was localized in both the rough endoplasmic reticulum (ER) and the Golgi apparatus. Later, the radioactivity over granules increased while that over the Golgi apparatus decreased. The concentration of silver grains over the dilated cisternae of the rough ER was not found to be modified at the longest time intervals studied.

Recent work on the synthesis of glycoproteins has demonstrated that the different sugars are added step by step to the preformed polypeptide chains, according to their position in the lateral side chains. Studies on the synthesis of thyroglobulin (Spiro and Spiro, 1966; Herscovics, 1969, 1970) and immunoglobulin (Swenson and Kern, 1968; Melchers, 1970; Schenken and Uhr, 1970) have shown that whereas glucosamine and mannose are incorporated during synthesis of the polypeptide chains, the terminal sugars, e.g., fucose, are added after the completion of the polypeptide backbone. Autoradiographic data have also shown that in thyroid cells (Haddad et al., 1971), hepatocytes,

and duodenal cells (Bennett and Leblond, 1970, 1971), fucose is incorporated into glycoproteins in the Golgi apparatus. In rat anterior pituitary in vitro, incorporation of mannose and glucosamine into total glycoproteins was prevented by puromycin within 30 min whereas the addition of puromycin to the incubation medium did not decrease fucose incorporation before 45 min of incorporation (Todd and Samli, 1973). In the stimulated thyrotrophs (thyroidectomy cells) of the rat adeno-hypophysis, fucose was found to be incorporated directly in both the rough endoplasmic reticulum (ER) and the Golgi apparatus (Pelletier and Puviani, 1973). No results are available on the migration of newly formed glycoproteins in the different adeno-hypophyseal cell types. Thus, it appeared of interest to follow in vivo the intracellular pathways of newly synthesized glycoproteins both in cells which secrete and in cells which do not secrete glycoprotein hormones. It also seemed important to localize the incorporation of [³H]fucose in stimulated gonadotrophs (gonadectomy cells) which secrete glycoprotein hormones (LH and FSH) and to study the migration of glycoproteins in this hypersecreting cell.

MATERIALS AND METHODS

Sherman male rats weighing about 40 g received 5 mCi of L-[³H]fucose (specific activity 4.3 Ci/mmol). Animals were sacrificed 5 min, 20 min, 1 h, and 4 h after the injection by cardiac perfusion of the fixative. The fixative used was 2.5% glutaraldehyde in a 0.05 M Sorensen buffer containing 0.1% sucrose and 1% fucose. After perfusion, the tissue was kept in the same fixative for 2 h.

Gonadectomy cells were studied in male rats which had been castrated 1 mo previously. Since these animals weigh about 200 g, a large amount of radioactive material would have been required for in vivo experiments and so, instead, pituitaries were incubated in vitro. Pituitary fragments were pulse labeled for 10 min in a Krebs-Ringer buffer solution (for details on the incubation procedure, see Pelletier et al., 1972) containing 1 mCi/ml of [³H]fucose and incubated postpulse in a medium containing 10 mM cold fucose for further periods of 1 h and 4 h. The tissue was then fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer.

In all cases, the pituitary tissue was postfixated for 2 h in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Epon or Araldite. Semithin (1 μm) sections were mounted on glass slides and coated with Kodak NTB2 nuclear emulsion. After appropriate exposure, autoradiographs were developed and poststained with toluidine blue. For a better identification of cell types, some sections were stained with orange G-periodic acid-Schiff

(PAS) after removal of the Epon before being processed for autoradiography. Ultrathin sections showing silver interference color were autoradiographed with diluted Ilford L-4 according to the technique of Whur et al. (1969).

For light microscopy, the grain counts were performed after a 10-day exposure. For each time interval, the number of silver grains were counted over at least 100 somatotrophic or gonadotrophic cells. At the electron microscope level, the grains were counted on photographic enlargements (final magnification × 30,000). As described by Whur et al. (1969), Haddad et al. (1971), and Nadler (1971), each silver grain was circumscribed within a circle of 6.7-μm diameter. The source of radioactivity has been calculated to be localized in this circle with 95% probability. If only one organelle was found within the circle, it was classified as "exclusive." When more than one organelle were localized within the circle, the grain was classified as "shared." Using the method of Nadler (1971), the shared grains were attributed, on the basis of probability, to only one structure. The corrected grains for each organelle were the sum of exclusive and adjusted shared grains and were expressed as a percentage of the total number of corrected grains (Tables II, III, IV). At least 400 grains were analyzed for each time interval.

RESULTS

In Vivo Incorporation of [³H]Fucose

LIGHT MICROSCOPE AUTORADIOGRAPHY: All the cells present in the anterior pituitary showed an incorporation of labeled fucose. At 5 min after injection of fucose, a few grains were concentrated over a small area of the cytoplasm. At longest time intervals, the grains were dispersed over the cytoplasm. In both somatotrophs (orange G positive) and gonadotrophs (highly PAS positive), the number of silver grains overlying the cytoplasm of these two cell types did not appear to be modified significantly from 20 min to 4 h (Table I).

TABLE I
Grain Counts over Somatotrophic and Gonadotrophic Cells in Light Microscope Autoradiographs after Injection of [³H]Fucose*

Time after injection	Somatotrophic cells	Gonadotrophic cells
5 min	2.8 ± 0.7	4.1 ± 1.1
20 min	4.5 ± 0.9	9.2 ± 2.4
1 h	5.0 ± 1.1	8.6 ± 1.6
4 h	5.4 ± 0.8	9.7 ± 2.0

* Mean ± SEM.

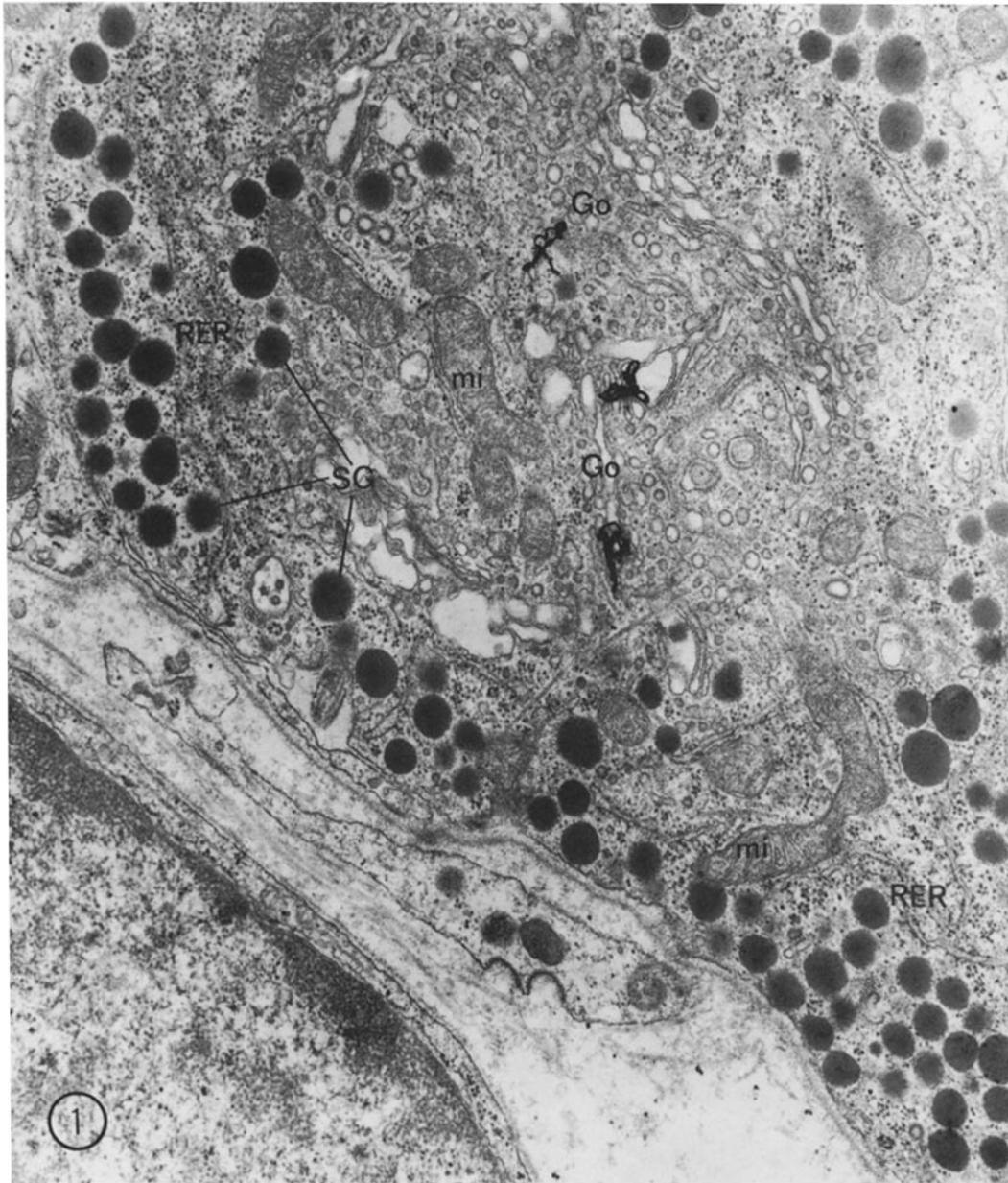


FIGURE 1 Autoradiograph of a somatotroph 5 min after injection of [^3H]fucose. Silver grains are localized over the Golgi apparatus (*Go*). Secretory granules (*SG*), rough ER (*RER*), and mitochondria (*mi*) are free of radioactivity. $\times 24,000$.

ELECTRON MICROSCOPE AUTORADIOGRAPHY: The distribution of autoradiographic grains was analyzed in both polypeptide- and glycoprotein-secreting cells. Somatotrophs (growth hormone-secreting cells), which represent a large proportion of adenohypophyseal cells, were selected as the polypeptide hormone-secreting cells and gonadotrophs (LH- and FSH-secreting cells) as the glycoprotein-secreting cells. The somatotrophic cell is a round or oval-shaped cell charac-

terized by the presence of secretory granules which are dense and round and have a diameter of 250–350 nm (Kurosuni, 1968; Farquhar, 1971). The rough ER usually occurs in the form of parallel lamellae (Figs. 1–3). The gonadotroph is a larger cell with a round shape and a rough ER which is more dilated than that of the somatotroph (Figs. 4, 5) (Kurosuni, 1968; Farquhar, 1971). It

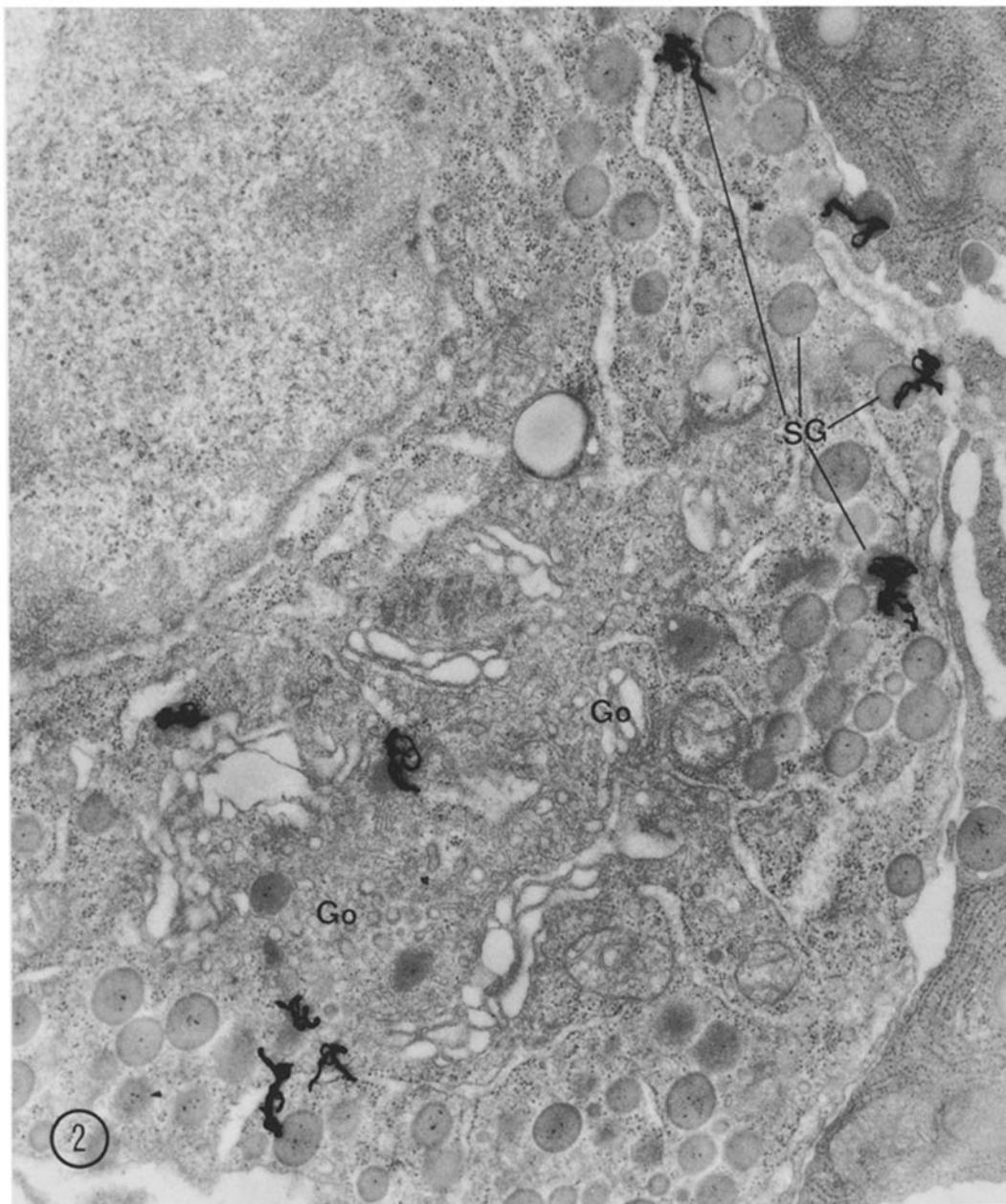


FIGURE 2 Autoradiograph of a somatotroph 20 min after injection of [³H]fucose. Autoradiographic grains appear mainly over secretory granules (SG), most of these labeled granules being located at the periphery of the cell. Some label is still associated with the Golgi complex (Go). $\times 23,100$.

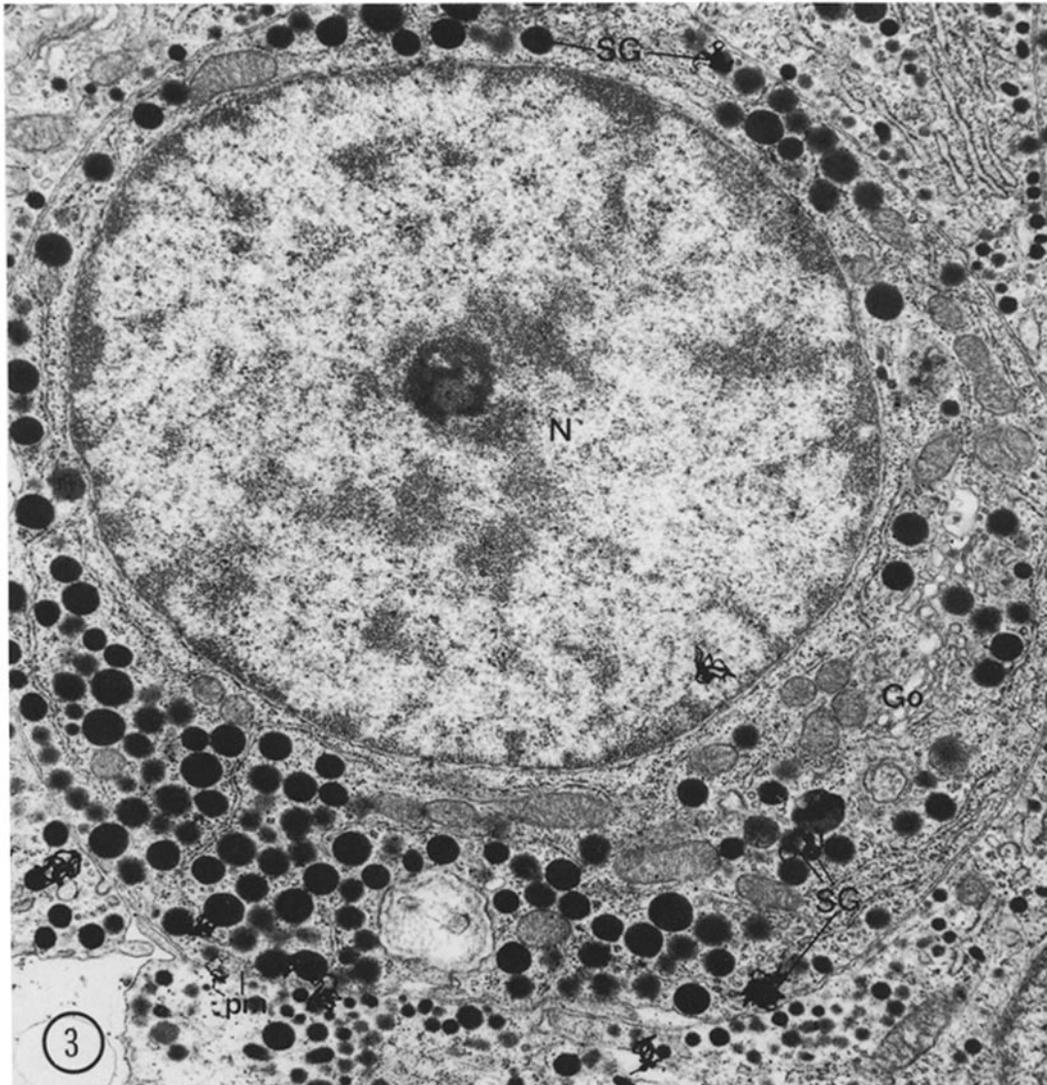


FIGURE 3 Autoradiograph of a somatotroph 4 h after injection of [^3H]fucose. With the exception of one grain found over the nucleus (N), all the grains overlie secretory granules (SG) or the plasma membrane (pm). The Golgi apparatus and the rough ER are not labeled. $\times 16,300$.

contains both small (150–250 nm) and large (400–600 nm) granules. This cell type has been shown to contain both LH and FSH (Nakane, 1970; Pelletier, unpublished data).

The distribution of silver grains in somatotrophs is indicated in Table II. In the somatotroph, 5 min after the fucose injection, as much as 80% of the radioactivity was localized over the Golgi apparatus (Fig. 1). Usually both Golgi saccules and Golgi vesicles contained labeled fucose. The concentra-

tion of autoradiographic grains over the Golgi apparatus was still marked at 20 min, but many grains were associated with secretory granules, most of them being located at the periphery of the cell (Fig. 2). At 1 h and 4 h, almost no labeling of the Golgi apparatus was observed (Fig. 3). Most of the labeled glycoproteins were localized in the granules. Lysosomes, which were not abundant in these young animals, were occasionally labeled. At 20 min, 1 h, and 4 h, some radioactivity was

inconsistently associated with mitochondria and nuclei. These structures were ignored in the analysis of grain distribution. At the longest time intervals (1 h and 4 h), grains were frequently observed over the plasma membrane without being associated with the secretory granules. Since the plasma membrane is not a large enough structure to have exclusive grains, it has been excluded from the grain count distribution. Nevertheless, it seems reasonable to suppose that when there is no closely associated granules, some grains could arise from the plasma membrane, since the external part of the plasma membrane is usually in contact with the extracellular space whereas the inner portion is usually in contact with the rough ER which is usually weakly labeled.

In the gonadotroph, the incorporation of fucose was very similar to that observed in the somato-

troph (Table III). At the 5-min interval, silver grains were localized almost exclusively (90%) over the Golgi apparatus (Fig. 4). By 20 min, 50% of grains were still present over the Golgi apparatus. Secretory granules in the Golgi area were also found to be labeled. At longer time intervals (1 h and 4 h), most labeled glycoproteins were found in the secretory granules (Fig. 5). Both the large and small secretory granules were found to be labeled (Fig. 6). As in the somatotroph, some grains were consistently associated with the plasma membrane. Lysosomes, mitochondria, and nuclei were very occasionally found to contain some radioactivity.

By 4 h, silver grains were often seen in the dilated extracellular spaces containing sparse fibrillar material (Fig. 7). At this time interval, an accumulation of silver grains in the basement

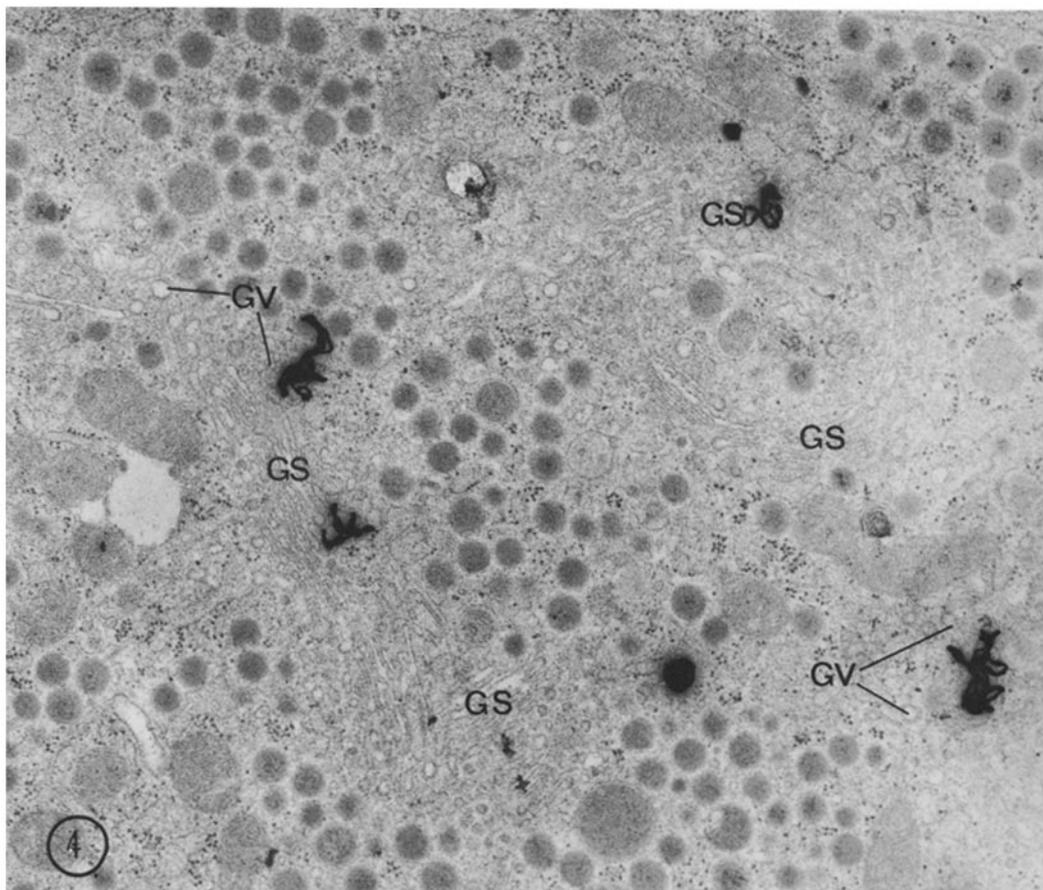


FIGURE 4 Autoradiograph of a gonadotroph 5 min after injection of [3 H]fucose. The autoradiographic grains are primarily located over elements of the Golgi complex, underlying both Golgi saccules (GS) and Golgi vesicles (GV). $\times 27,200$.

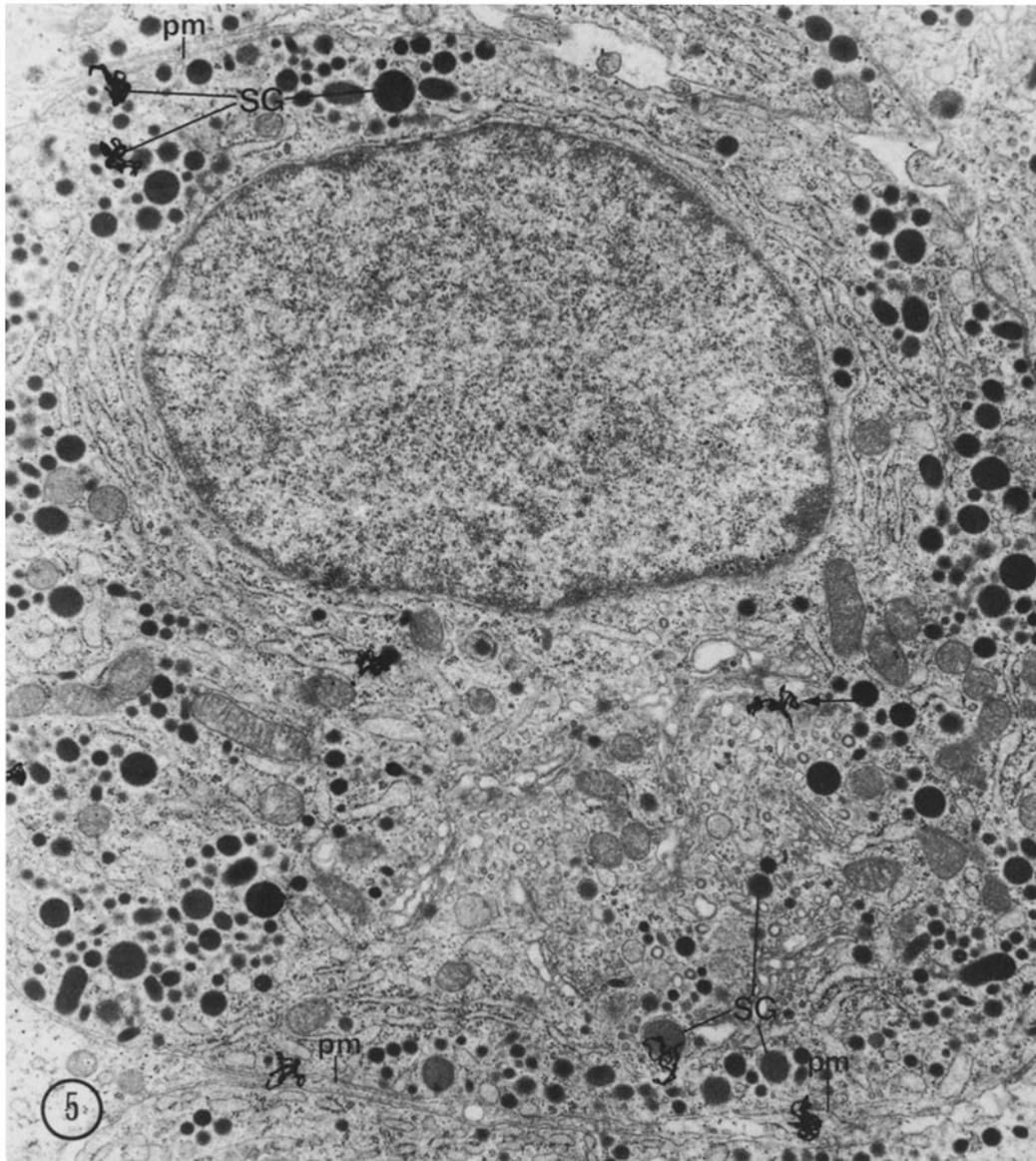


FIGURE 5 Autoradiograph of a gonadotroph 1 h after the injection of [^3H]fucose. One silver grain appears over the Golgi apparatus (arrow). The other grains are associated with the secretory granules (SG). pm, plasma membrane. $\times 13,000$.

membrane was also frequently noted (Fig. 7). Endothelial cells and fibroblasts were usually labeled at all time intervals.

In Vitro Incorporation of [^3H]Fucose

LIGHT MICROSCOPE AUTORADIOGRAPHY: The gonadectomy cells were easily identified as large cells containing very dilated cisternae and large number of PAS-positive granules. The number of

silver grains, distributed quite evenly in the cytoplasm, did not vary as a function of time. An average of 15 grains per cell was recorded over the gonadectomy cells at any time interval.

ELECTRON MICROSCOPE AUTORADIOGRAPHY: The enlarged gonadectomy cells were characterized by the presence of both dilated cisternae of the rough ER and a hypertrophied Golgi apparatus (Figs. 8, 9). The secretory granules,

which are usually abundant, were not found in the cisternae. At the end of the 10-min pulse-labeling period, about 43% of the grains were localized over the Golgi apparatus and 45% over the cisternae of the rough ER (Table IV and Fig. 8). After 1 h of postpulse incubation, most silver grains were found over cisternae of the rough ER (40%) and secretory granules (46%). By 4 h, the distribution of radioactivity was approximately the same as that after the 1-h interval, most grains being related to cisternae of the rough ER and the secretory granules of both types (Fig. 9). The

grains corresponding to cisternae had no special localization and were found in the center, as well as in the periphery, of the cisternae. As observed in the *in vivo* experiments, a few nuclei, mitochondria, and lysosomes were labeled after 1 h and 4 h of chase incubation.

DISCUSSION

Since there is no retention of free [³H]fucose after glutaraldehyde fixation (Haddad et al., 1971; Pelletier and Puviani, 1973) and since fucose is incorporated as such into glycoproteins without

TABLE II
Relative Concentration (Percent Corrected Grain Count) of Label Per Organelle of the Somatotroph after the Injection of [³H]Fucose

Time after intravenous injection	5 min	20 min	1 h	4 h
Rough ER	10	14	12	13
Golgi complex	80	52	20	16
Secretory granules	10	34	68	71

TABLE III
Relative Concentration (Percent Corrected Grain Count) of Label Per Organelle of the Gonadotroph after the Injection of [³H]Fucose

Time after intravenous injection	5 min	20 min	1 h	4 h
Rough ER	6	12	9	9
Golgi complex	90	50	22	20
Secretory granules	4	38	69	71

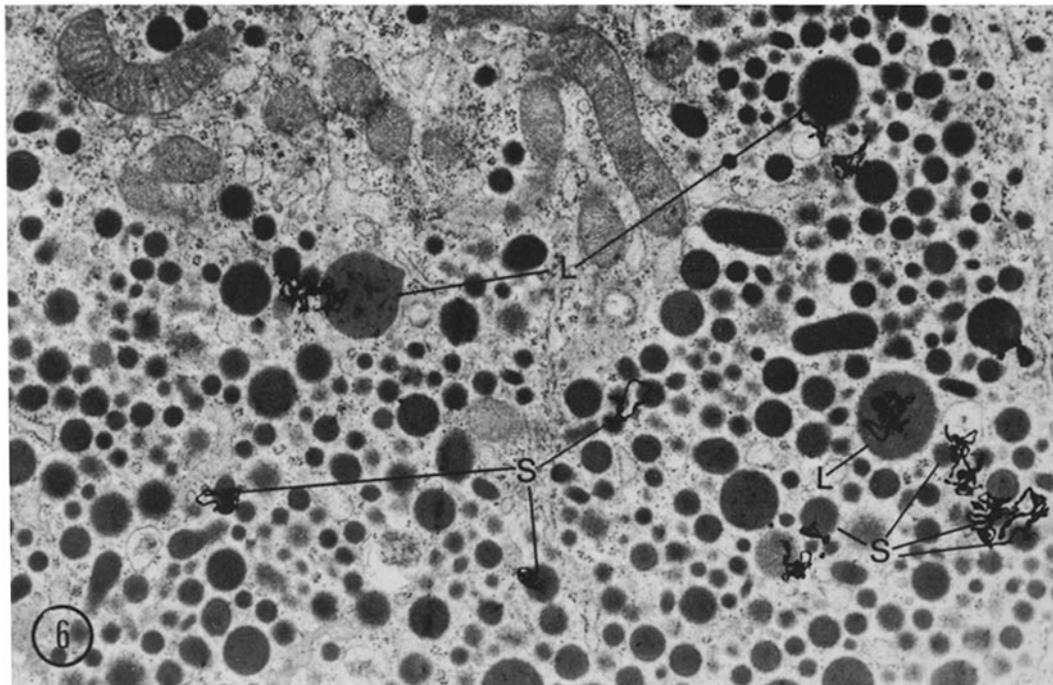


FIGURE 6 Autoradiograph of a gonadotroph 4 h after injection of [³H]fucose. The autoradiographic grains are located mainly over both the large (L) and the small (S) secretory granules. $\times 20,100$.

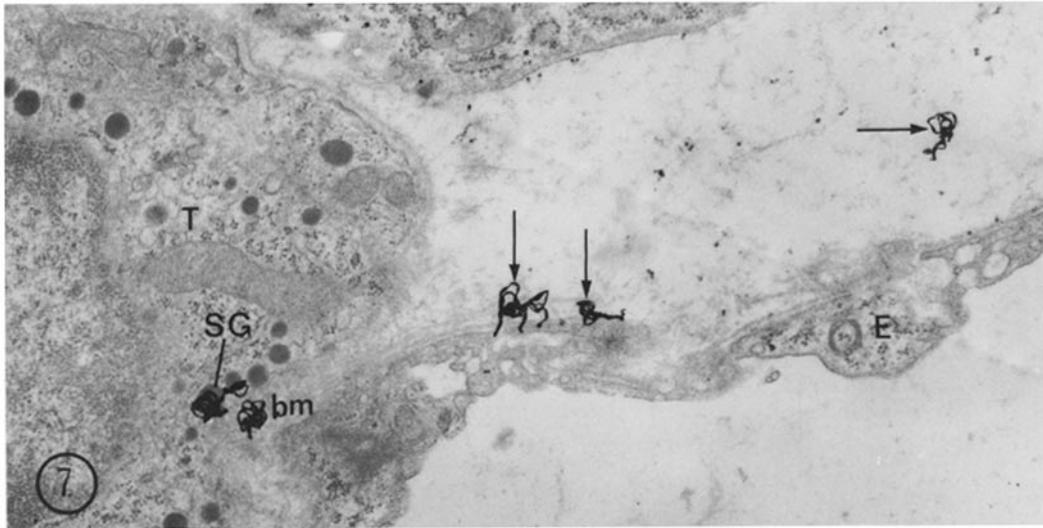


FIGURE 7 Portion of a perivascular space 4 h after injection of [^3H]fucose. The label appears over secretory granules (SG) of a thyrotroph (T), basement membrane (bm), and is also associated with fibrillar material (arrows) found between the endothelial cell (E) and the secretory cells. $\times 18,000$.

previous conversion into other compounds (Coffey et al., 1964; Bekesi and Winzler, 1967; Herscovics, 1970), it is very likely that silver grains observed in these experiments represent newly synthesized glycoproteins. In our experiments, a doubling of [^3H]fucose incorporation into gonadotrophs and somatotrophs has been observed between 5 and 20 min after the intravenous injection of the labeled sugar. Haddad et al. (1971) have also found a 2.5-fold increase in the incorporation of radioactivity into thyroid follicular cells from 5 to 20 min after the injection of [^3H]fucose. These data suggest that an appreciable amount of [^3H]fucose remains in the circulation for more than 5 min or that radioactivity already present inside the cells is incorporated into glycoproteins between 5 and 20 min after injection, although a combination of both phenomena is possible. In fact, Bocci and Winzler (1969) have reported that 10 min after intravenous injection of [^{14}C]fucose, 10% of the injected dose of radioactivity was still present in the circulating blood. As the number of silver grains did not vary from 20 min to 4 h, the kinetic analysis of glycoproteins was considered valid. As previously observed in hepatocytes (Bennett and Leblond, 1971), duodenal (Bennett and Leblond, 1970), and thyroid cells (Haddad et al., 1971), the [^3H]fucose was incorporated directly into the

Golgi apparatus in both somatotrophs and gonadotrophs after intravenous injection of the labeled sugar.

Todd and Samli (1973) had observed that inhibition of protein synthesis by puromycin for 45 min did not affect the incorporation of fucose into glycoproteins of rat adenohypophysis. These observations and the present findings strongly suggest that fucose, a terminal sugar of glycoproteins (Spiro, 1970), is added to preformed polypeptide chains which have already migrated to the Golgi apparatus in the somatotroph and the gonadotroph. This is very similar to what has been observed for the biosynthesis of thyroglobulin (Herscovics, 1970; Haddad et al., 1971).

It is important to note that the somatotroph which secretes a polypeptide hormone has the same characteristics with respect to incorporation and fate of the labeled fucose as the gonadotroph which produces glycoprotein hormones. The transport of fucose-labeled proteins to the secretory granules of somatotrophs suggests that some glycoproteins are a constituent of the matrix of the granule and/or the granule membrane. In both cell types studied, the migration of glycoproteins from the Golgi apparatus to the granules is apparent as soon as 20 min after fucose injection and is maximal at 1 h. These results are in agreement

with those obtained in somatotrophs and mammothrophs after labeling of proteins with [^3H]leucine, which have shown migration of proteins from the Golgi apparatus to the granules within 30 min (Howell and Whitfield, 1973; Labrie et al., 1973).

Although, as a consequence of the poor resolution of autoradiography, no labeling of the plasma membrane can be definitely stated to occur, it seems likely that the regular occurrence of label over the plasma membrane is due to the presence

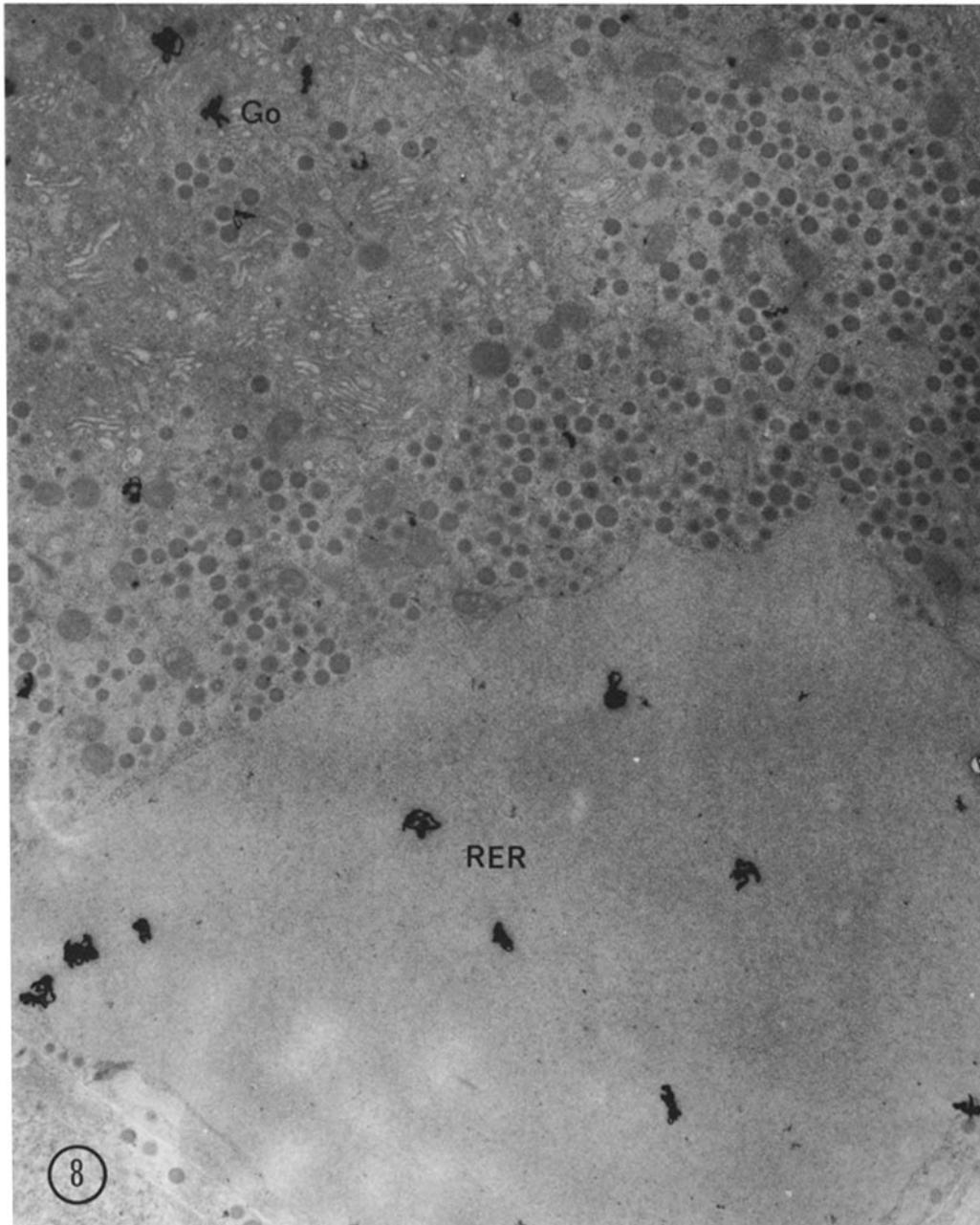


FIGURE 8 Autoradiograph of a gonadectomy cell from a pituitary fixed after a 10-min pulse-labeling incubation with [^3H]fucose. Silver grains are concentrated mainly over both the Golgi complex (*Go*) and the cisternae of the rough ER (*RER*). $\times 13,400$.

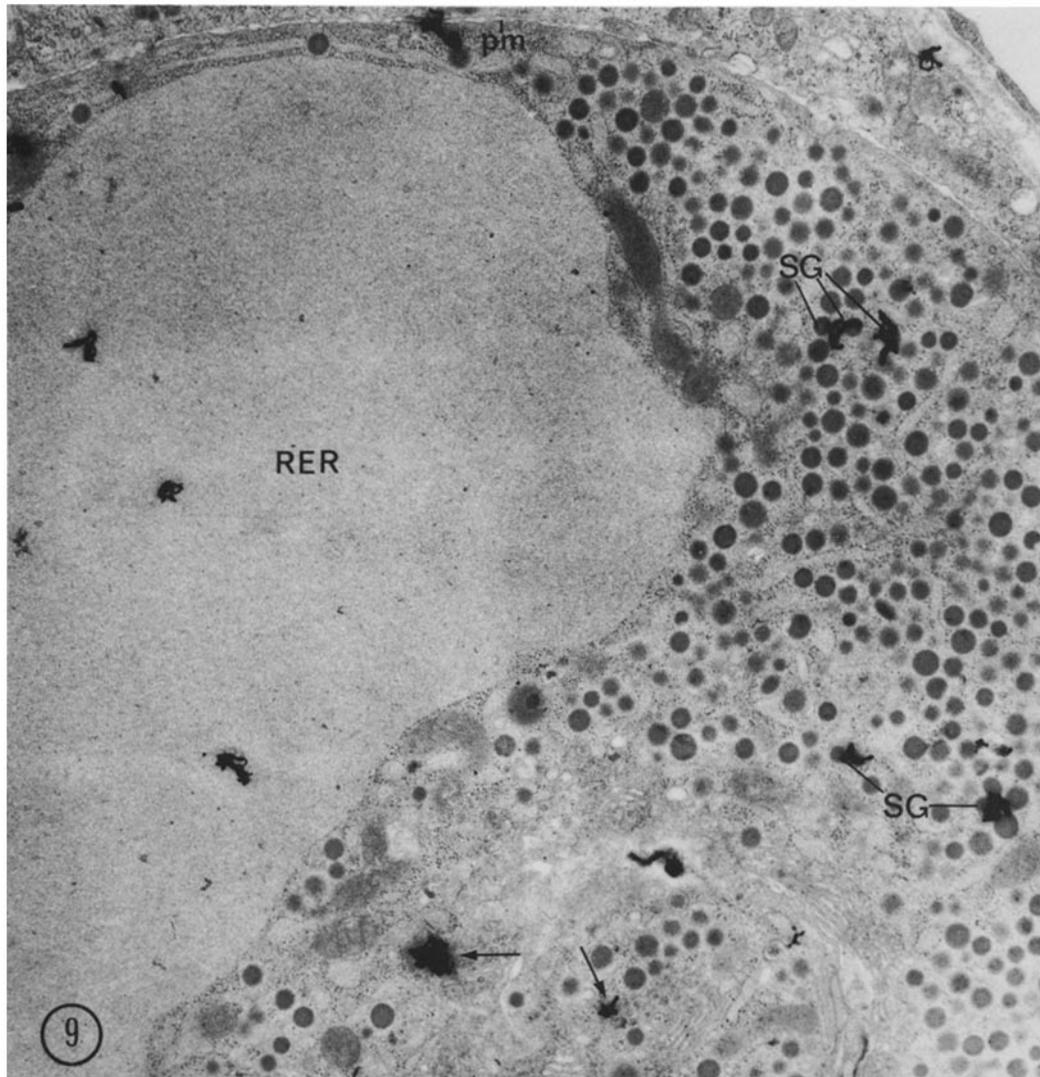


FIGURE 9 Autoradiograph of gonadectomy cell pulse labeled 10 min in a medium containing [^3H]fucose and incubated postpulse for 1 h in chase medium. The grains are located over secretory granules (SG) and dilated cisternae of the rough ER (RER). Some label is still associated with elements of the Golgi complex (arrows). pm, plasma membrane. $\times 16,000$.

TABLE IV
Relative Concentration (Percent Corrected Grain Count) of Label Per Organelle of the Gonadectomy Cell after Pulse Labeling with [^3H]Fucose

Duration of incubation after 10 min pulse labeling	0	1 h	4 h
Rough ER	45	40	37
Golgi complex	43	14	12
Secretory granules	12	46	51

of newly synthesized glycoprotein(s) in the membrane itself. The presence of glycoproteins in the plasma membrane of pituitary cells detected by histochemical techniques has been reported (Rambourg and Leblond, 1967; Pelletier, 1971). Bennett and Leblond (1970) have also reported substantial labeling of the plasma membrane of the columnar cells of the small intestine after the injection of labeled fucose.

The presence of newly synthesized glycoproteins

in the basement membrane also suggests a continuous synthesis of the glycoproteins which are constituents of the basement membrane. The radioactivity found in the extracellular space could belong to some glycoproteins present in the fibrillar extracellular material or could be related to some release processes, for example, the release of glycoprotein hormones. Todd and Samli (1973) have reported that 4% of all fucose-labeled proteins were released into the incubation medium 30 min after the incorporation of the labeled sugar.

In the gonadectomy cells, the simultaneous incorporation of [³H]fucose into the dilated cisternae of the rough ER and the Golgi apparatus is similar to previous findings in the thyroidectomized cells (Pelletier and Puviani, 1973). These results clearly indicate that, under conditions of extensive stimulation of secretion, glycoprotein-secreting cells (gonadotrophs and thyrotrophs) incorporate fucose not only in the Golgi apparatus but also in the rough ER. A fucosyltransferase would, presumably, be present in both Golgi apparatus and rough ER. As it is likely that enzymes of the Golgi apparatus are synthesized in the rough ER, it may be suggested that they have been accumulating in the cisternae of the rough ER where they have some activity. From these studies, it is not possible to know if gonadotrophic hormones (LH, FSH), which have been found to contain fucose (Jutisz and De La Llosa, 1972), are synthesized in the rough ER. The extensive hypertrophy of the Golgi apparatus and the migration of labeled glycoproteins into the secretory granules suggest that gonadotrophic hormones are probably released after a previous storage in the secretory granules. On the other hand, the persistent labeling of the cisternae of the rough ER suggests that newly formed glycoproteins are retained in the cisternae or have a slow rate of migration to other organelles.

The assistance of Dr. Antonio Haddad who did the injection of [³H]fucose in the laboratory of Dr. C. P. Leblond is acknowledged. We express our thanks to Mr. Romano Puviani for his excellent technical assistance.

This research work was supported by grant MA-4242 from the Medical Research Council of Canada. G. Pelletier is a Scholar of the Medical Research Council of Canada.

Received for publication 28 August 1973, and in revised form 24 January 1974.

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